Saskatchewan
Agriculture
and Food

ADF

AGRICULTURE DEVELOPMENT FUND

FINAL REPORT

20030530

ADDING VALUE TO LENTILS THROUGH IMPROVEMENT OF VISUAL QUALITY CHARACTERISTICS

Funded by: The Agriculture Development Fund

March 2008

Prepared by: University of Saskatchewan (U of S)

Adding value to lentils through improvement of visual quality characteristics

March 24, 2008
Final Report

Research funded by:

Saskatchewan Agriculture & Food Agriculture Development Fund
and
Saskatchewan Pulse Growers

Investigators:
Kirstin Bett, Abebe Tullu and Bert Vandenberg

Collaborators:
Blaine Davey, Adam Fritzler

Department of Plant Sciences, University of Saskatchewan

Project #: 20030530
MIDAS#:000306

A. Abstract

Lentil quality in the form of green colour retention and plumpness were investigated in this study. A method for assessing green colour in lentil was developed using a DuPont® Acurum® machine. RILs from a cross between a good quality green line and a lower-quality line were assessed in multiple environments. The distribution of 'greenness' score for these RILs suggests that the trait is quantitatively expressed thereby necessitating a QTL approach to marker development. A major QTL was identified that explains approximately 20% of the variability for green colour retention. RILs from a population segregating for roundness were phenotyped using slotted and round hole screens and this trait was also found to be quantitative in nature. Molecular marker analysis on the entire data set did not reveal any major QTL. Further marker screening and increased phenotyping will be necessary to more accurately define this QTL. Based on this research, changes have been made to the lentil breeding program to enhance the ability to select for green colour retention and plumpness.

B. Executive Summary

Lentil seed quality is largely dictated by the appearance of the seed. For green lentils, the ability of the seed to have and maintain good green colour is important. For red lentils, the shape is becoming important as millers prefer plumper material as it increases the milling efficiency.

Green lentil genotypes with improved colour retention have been identified and are being used in the breeding program to improve the overall green quality of the lentil seed coats. By quantifying greenness over a range of genotypes (cultivars and recombinant inbred lines (RILs)) we have ascertained that it is a quantitative trait with a large component of environmental variation but that the genetic control appears to be fairly simple. There is genetic variability for greenness within the breeding program and new lines coming out of the program have improved colour. Molecular marker analysis of a population segregating for greenness demonstrated a major QTL for improved colour. The markers associated with this QTL are AFLP-based so will either have to be converted to a SCAR or new markers from new EST mapping projects that map to this region will have to be identified.

A study of the effect of swathing vs desiccation as a pre-harvest treatment on the greenness of a variety demonstrated that swathing in general was preferable; however, there is an increased risk of weathering in the swath that must be managed to take full advantage of this technique for maintaining green colour in the seed coats.

Phenotyping plumpness in red lentil is challenging. As the seeds are lens shaped, orbital sorters were not fully effective in distinguishing differences in plumpness. As an alternative, we used slotted and round hole screens to assess the thickness and diameter of samples of different genotypes. Thickness of the seed is one indicator but it is confounded by the diameter of the seed since larger seeds are probably thicker but not necessarily plumper. An index of diameter to thickness ratio was developed to better describe the plumpness. Assessment of a RIL population derived from a cross between a plump Turkish lentil genotype and a less plump breeding line demonstrated transgressive segregation for both thickness and diameter. Both of these characteristics appeared to be quantitatively inherited as was the index. Molecular marker analysis of this population failed to reveal any QTL associated with thickness, diameter or the index although one region on one linkage group was significant when only the 10 smallest diameter lines were compared to the 10 largest lines. More markers and phenotyping of more individuals will hopefully

change this. Alternatively a better method of phenotyping may be required to more accurately identify regions of the genome related to plumpness.

This project has allowed us to gain a better understanding of the genetics of these traits and selection practices in the breeding program have been altered to better assess these characteristics in segregating generations. The outcome has been, and will continue to be, new green lentil varieties with better green colour at harvest and plumper red lentil varieties for increased milling efficiency.

C. Technical Report

Introduction

Quality of the lentil crop, like that for most pulses, is based primarily on visual characteristics of seeds such as colour, size and shape. For the green lentil market class, seed coat colour retention is influenced by environmental factors during harvest and storage. Little is known about the genetics of colour retention. Seed size distribution and shape influence the market value of lentils, especially reds. We were interested in determining if improved seed coat colour retention for green lentil and rounder seed shape for red lentil can be bred into well-adapted varieties through effective screening techniques and efficient use of molecular markers.

This project consisted of three major objectives:

1) To determine the basis for genetic control of colour retention in the seed coats of green lentil varieties;
2) To determine the basis for genetic control of round seed shape in red lentil; and
3) To identify markers for these two traits to assist in genetic improvement efforts to develop better quality lentil varieties.

The retention of green seed coat colour is important because the crop discolours and loses value with colour change caused by weathering and aging, especially when combined with conditions that combine heat, humidity and light. We have recently identified breeding line 1294m-23 as a source of exceptional green colour retention.

The shape of red lentils ranges from a flatter, lens shape (e.g. cv. Crimson) to a much rounder, "football"shape, typical of Turkish lentils. In most lentil markets around the world, red cotyledon types are consumed after the seed coat is removed but the cotyledons are not split. In Bangladesh and north-east India, split lentils have traditionally been a byproduct of the decortication process and a premium is paid for unsplit product. Turkish lentil processors adopted and modified the decortication technology to expand markets for unsplit decorticated lentils, now commonly known as the "football" type. The preferred shape for processing is as round as possible to improve product appearance, and to maximize recovery during processing. A survey of 36 lines from three environments in 2003 showed that seeds of small-seeded lines ranged from less than 10% round to over 50% round (based on results of orbital sorting). Results were relatively consistent across locations (unpublished data). In a 2002 Pulse Canada market study, most of Turkish lentil market samples from Mersin were classified as 100% round using an orbital sorter. This suggests genetic control of the trait rather than just environmental effects. Lentil breeders in Australia and the USA have also hypothesized that round seed shape is under relatively simple genetic control. This research involves investigating the genetic basis for roundness and the potential for incorporating it

into the breeding program using molecular markers as a strategic supplement to the conventional breeding techniques.

Research results in detail:

1. Basis for genetic control of colour retention in the seed coats of green lentil

Samples of 100 seeds each for 36 green lentil genotypes were colour-analyzed from eight trials grown throughout the commercial lentil production area of Saskatchewan in 2005. Four lines were commercially grown industry checks - CDC Sovereign, CDC Glamis, CDC Plato, and CDC Sedley. The remaining lines were advanced breeding lines. Trials were desiccated prior to harvest and threshed with a small plot combine at maturity. A sub-sample was drawn from the harvest samples of the first replication of each yield trial. Subsamples of these plots were characterized for colour using DuPont's Acurum technology. It categorizes colour by using an eight bit colour display to define the colour and then classifies each seed of the sample into one of 19 different colour bins using a neural net model based on human visual classification by experienced lentil graders. The resulting data were then indexed to provide an average bin # score for each entry. Lower index scores indicate that seed coats in the sample are "greener" (Fig. 1).

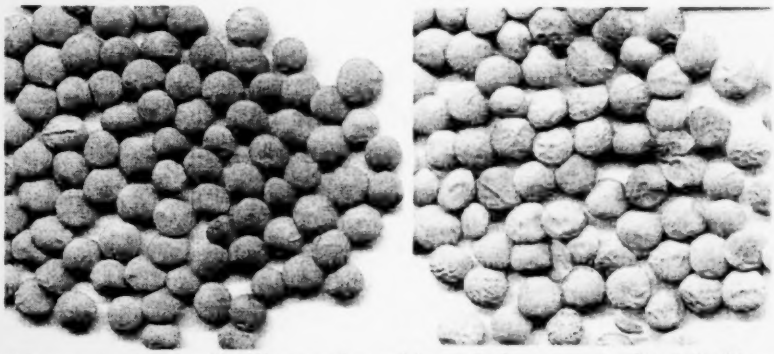

Figure 1. Green lentil samples with an index score of 2.6 (left) and 8.2 (right) on the Acurum scale.

We observed significant differences in index scores for green colour among the lines investigated (Fig. 2). The mean index across the environments was the lowest ("greenest") for breeding line 1294M-23. The index score for this line was significantly lower (p=0.05) than 26 of the 36 genotypes. The industry checks were statistically "less green" than 1294M-23. 1294M-23 is one of the parents in the RIL population used in the mapping portion of this project. The least green genotype was breeding line 1444S-7, although it was not statistically (p=0.05) less "green" than CDC Sovereign, CDC Plato or CDC Sedley. CDC Glamis was the only check that was statistically (p=0.05) more green than 1444S-7. Environment contributed a large variation in the index score (Fig. 3). Two sites caused most of the environmental variations: Davidson and Wilkie. Davidson had a late harvest while Wilkie received hail. Thus it appears that much of the variation is genetic.

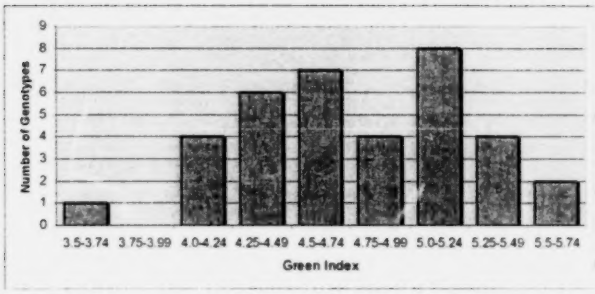

Figure 2. Frequency of 36 lentil genotypes in each green index category across eight environments.

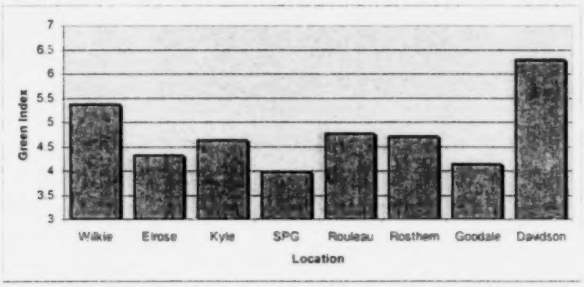

Figure 3. Mean green index of 36 genotypes for each location.

Replicated sets of a RIL population (LR06) segregating for green colour retention, plus the parents, were grown in the phytotron, a polyhouse, and in three field locations in 2005. The field locations were repeated in 2006 and all consisted of two replicates in an RCBD format. All 13 registered green lentil varieties were also included in the field trials. Subsamples of these plots were characterized for colour using DuPont's Acurum technology as described above.

There were no distinct index categories, rather a continuous range of index scores across the RILs (Fig. 4) and 12 of the 13 registered lines had indices at the "less green" end of the range. ANOVA using a mixed model showed that genotype was significant, suggesting that there is a genetic component to the variability in index scores. Location and year also contributed to a large part of the variability suggesting a heavy influence of the environment on this trait. Despite the contribution of the environment to the variability of this trait, the heritability was high (0.82) which means selection for better scores should lead to improvements in 'greenesss' of future lentil varieties. To best assess the genetic contribution to the index when breeding for improved colour, breeders should be assessing colour of samples from multiple locations and replications over at least two years to really sample the effect across different environments.

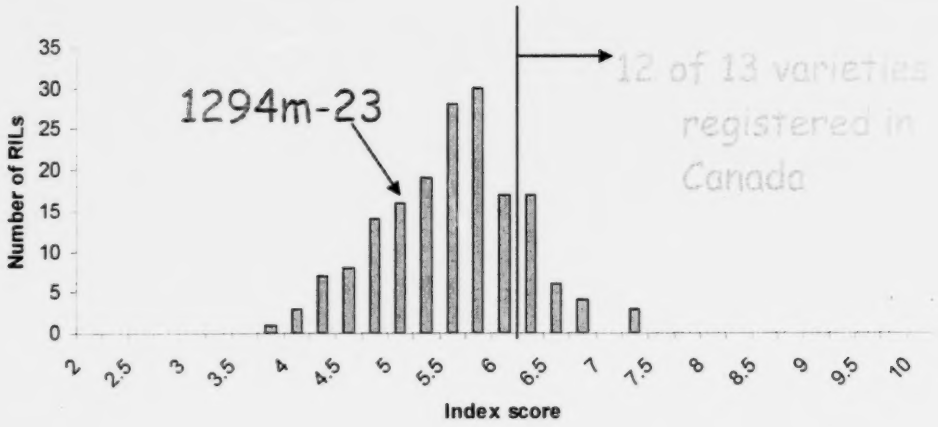

Figure 4. Frequency distribution of mean index scores for LR06 RILs based on samples from Elrose, SPG and Sutherland in 2005 and 2006. Where parent 1294M-23 would lie in this distribution is indicated. Lower index scores indicate better colour

The average field index score was not mirrored by results from the phytotron environment. The regression of the average field index score and the index score from the phytotron had an R^2 of 0.03. This indicates that indoor-grown plants cannot be used to predict which lines will have the most desired green colour phenotype in the field.

1.1. Additional agronomic data pertaining to the effect of the environment on green seed coat colour.

Fifteen green lentil genotypes, including all that are commercially available, were seeded at 2 sites near Saskatoon in 2005 and 2006. One site in each year was seeded later than the other to produce two different environments within the same year. The plots were swathed or desiccated with Reglone® when the bottom one third of the pods on the plant rattled when shaken. This is considered the proper stage for desiccation. After the plots were harvested the colour of the seeds was analyzed using the Acurum® machine from DuPont®. This system categorizes each seed into one of 19 predetermined colour categories based on standard lentil grading practices. Under this classification system, the lower the score, the "greener" the sample is.

Data collected over the two years shows that swathing the crop instead of desiccating with Reglone® produces a statistically "greener" sample (Fig. 5). This could be due to less light infiltration into the swath and a corresponding reduction in the loss of color. When large amounts of rain fell on the swaths, however, outbreaks of ascochyta and botrytis occurred and seed coats were negatively impacted. Some level of seed cleaning would be necessary in these instances to maintain a high grading lentil. Although swathing produced a "greener" sample, they tended to be of poorer quality. Some genotypes were significantly "greener" than others, and the best color retention on commercially available varieties was exhibited by Laird, CDC Grandora, and CDC Glamis. The varieties with the poorest color retention were CDC Milestone, CDC Vantage, and CDC Sedley. The best color retention was seen for the breeding line 1294M-23. Clearly a combination of cultivar selection and proper harvest management procedures can lead to improved green lentil quality.

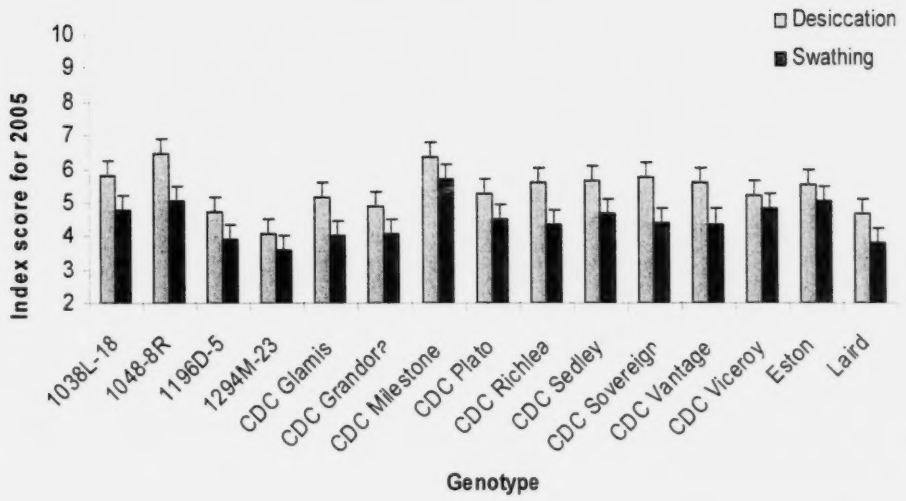

Figure 5. Mean index score of 15 genotypes subjected to pre-harvest treatment of desiccation or swathing. Error bars represent LSD ($\alpha = 0.05$).

2. Basis for genetic control of plumpness of red lentil seed

Based on a study of lines in the 2006 Lentil Regional Variety Trial we decided to use slotted and round screens to phenotype for roundness. Samples were taken from each entry, and processed through progressively smaller slotted sieves to establish an initial distribution based on minor diameter (thickness). In order to ensure genotype purity and to give a better idea of relative proportion samples were sieved a second time through successive round hole sieves in increments of $1/64^{th}$ of an inch and kept separate. Round hole fractions that occurred in a mass of greater than ten grams in any instance within a genotype were then sieved once more through slotted sieves. Slotted sieves were used in increments of 0.5/64" to establish a two-dimensional distribution. Twenty-four varieties were grown at 5 locations around SK. Both diameter and thickness were influenced by genotype and location ($p< 0.001$) but there was no interaction between genotype and location. The coefficient of regression (R^2) for thickness and diameter was 0.64 which agrees with the idea that the diameter has an influence on the thickness of a given seed.

Using just the slotted screens gives a measure of thickness but this is confounded by the size of the lentil; large lentil varieties are thicker, not because they are plumper but because they are larger in general. By calculating the ratio of width to thickness we can come up with a reasonable measure of plumpness. The two adjacent fractions which held the largest sum of seed by mass were determined and were designated the 'Index Fraction'. From the Index Fraction an Index Score was developed using the grouped mean diameter and thickness in millimeters. Dividing the grouped mean diameter by thickness and multiplying by a factor of 100 gave the Index Score.

Four lentil RIL populations were advanced to the F_6 stage and grown in the field in 2005 or 2006. RIL population LR44, from the cross LC8602303T x 1125-1-5, was the largest population, and was chosen for further phenotypic and molecular analyses. LR44 was grown in microplots in the field in Saskatoon in 2007. Seed samples of the RILs plus the parents were sieved through sets of slotted and round holed sieves to ascertain the distribution of seed shape within each line as described above.

The distribution of seed diameters was normally distributed suggesting quantitative inheritance (Fig 6). There was transgressive segregation for diameter with individual RILs having diameters beyond that of the parents. Only looking at the two data from the two most frequent screens resulted in a slightly different distribution but it did not help in determining the genetic control.

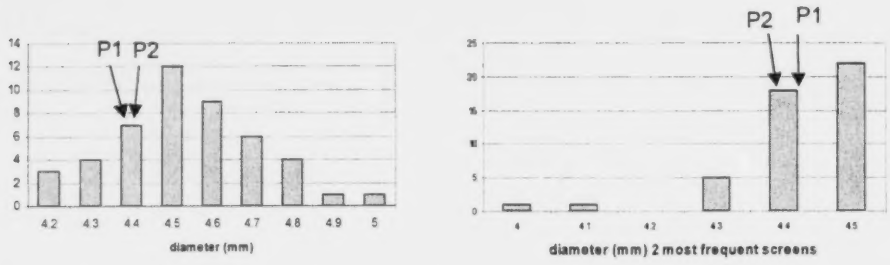

Figure 6. Frequency distributions of mean seed diameter for RILs. Scores for samples of P1 - LC8602303T and P2 – 1125-1-5 are indicated for reference. Distribution on the left represents average of all slotted screens while that on the right is the mean of the two screens that retained the most seed.

Another measure of seed diameter is simply to measure the percent of the seed sample that stays on a 6.5/64" screen. The distribution of this measurement across the RIL population appeared to be more of a bimodal distribution than a normal distribution suggesting single major gene control but still heavy influence from the environment and possibly other minor genes (Fig. 7).

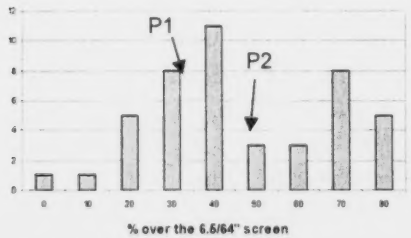

Figure 7. Frequency distribution of % seed sample retained above the 6.5/64" screen. Scores for samples of P1 - LC8602303T and P2 – 1125-1-5 are indicated for reference.

Seed thickness followed a normal distribution (Fig. 8), again with transgressive segregation and samples ranging from thinner to thicker than the parents. Again, quantitative inheritance is suggested by these results.

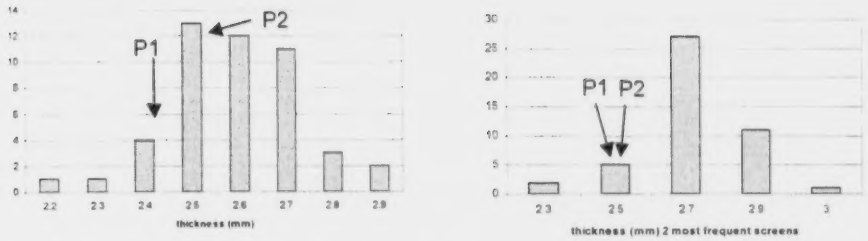

Figure 8. Frequency distributions of mean seed thickness for RILs. Scores for samples of P1 - LC8602303T and P2 – 1125-1-5 are indicated for reference. Distribution on the left represents average of all round hole screens while that on the right is the mean of the two screens that retained the most seed.

Seed thickness and diameter were correlated (r = 0.78; Fig. 9) confirming the hypothesis that seed diameter would confound the use of thickness to when trying to identify the plumpest seeds. There were, however individual lines that did appear to be thicker than predicted by the diameter, indicating plumper seeds. The confounding of thickness by diameter could be addressed through the use of an index based on the mean diameter and mean thickness for each RIL.

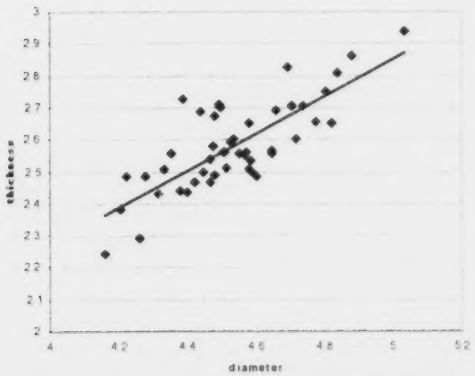

Figure 9. Correlation between thickness and diameter of the RILs from LR44. R = 0.78.

The index values for the individual RILs also followed a normal distribution. If only the two biggest fractions were considered though, there was more of a bimodal distribution (Fig. 10).

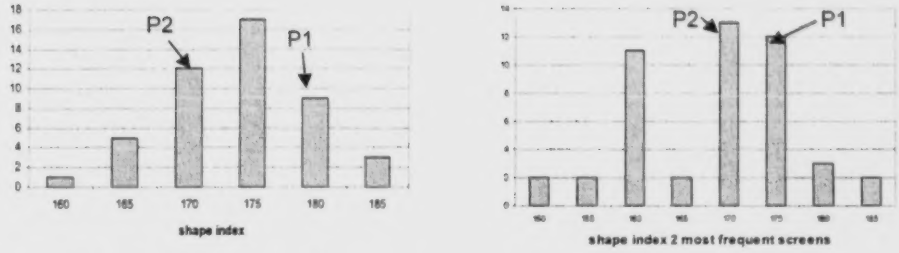

Figure 10. Frequency distribution of mean index value for RILs. Index scores for samples of P1 - LC8602303T and P2 – 1125-1-5 are indicated for reference. Distribution on the left represents the mean from all screens while that on the right represents the two screens that retained the most seed.

In summary, it would appear that seed shape is a quantitative character but it may be possible to identify single major genes that contribute to the majority of the variability in size within a sample.

3. Molecular genetic analysis of lentil populations segregating for green seed coat colour retention or plumpness

a) Colour

In addition to being phenotyped extensively, lentil RIL population LR06 was also used for molecular marker analysis to identify marker loci associated with retention of green seed coat colour. Parents, 1294m-23 and 1048-8R, were screened with 28 SSR markers and 13 different AFLP primer combinations. Of the 28 SSRs, three were polymorphic between the parents and were used to genotype 91 individuals from the mapping population. Six AFLP primer combinations resulted in between three and 12 polymorphic bands each and were used for mapping with 121 individual RILs. The others were either monomorphic or too difficult to see distinct polymorphisms.

Linkage analysis on the mapping data resulted in the identification of seven linkage groups and ten unlinked loci. As the markers were predominantly AFLP-based, it was not possible to relate specific linkage groups to previously published lentil molecular genetic maps. There is a good chance, however that the seven groups (LG01 to LG07) could correspond to the seven chromosomes in lentil.

Phenotypic results for QTL analysis were derived from field screening results at three different locations in each of 2006 & 2007 reported above. Phenotypic data from the screening in the phytotron were also included in the QTL analysis. Analysis revealed a significant QTL for green seed coat colour on LG04 in three of the six field locations: Sutherland 2005 and 2006 and Elrose 2006 (Fig. 11). This region accounted for 22%, 20.4% and 15% of the variability in greenness of the samples grown at Sutherland 2005 and 2006 and Elrose 2006, respectively. In all cases, presence of the allele from the greener parent (1294m-23) resulted in a shift in the index of about 0.5 units. Analysis of phenotypic data from the other three locations did not reveal significant regions associated with variability in colour scores.

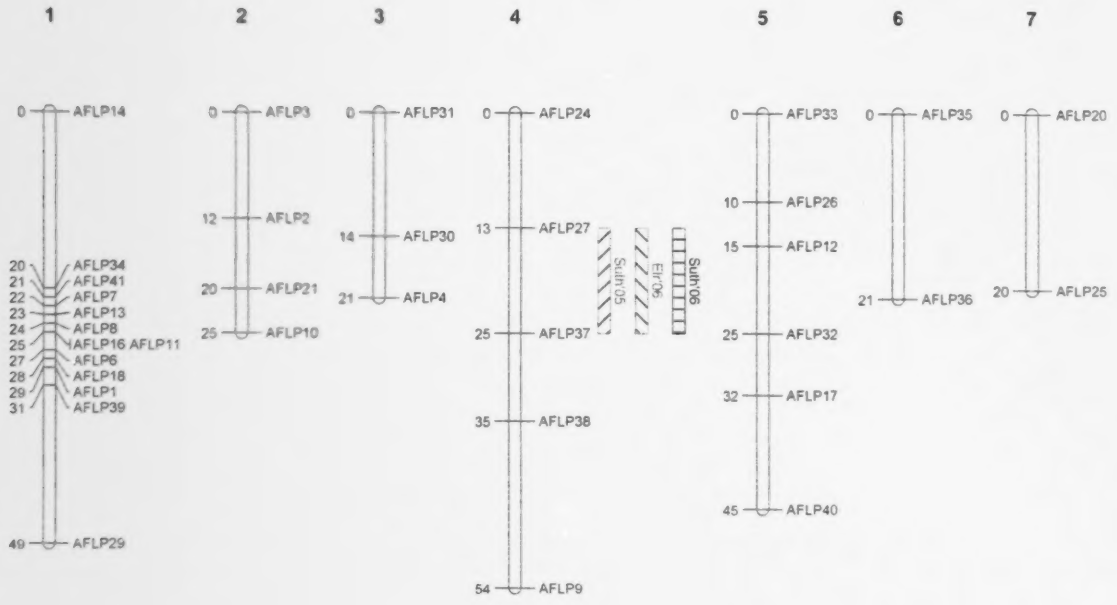

Figure 11. Genetic linkage map of LR06. Bars to the right of linkage group 4 indicate the region of the genome significantly ($p>0.95$) associated with green colour in three test locations.

Interestingly, Elrose 2006 was the site with the smallest range in phenotypic scores and Sutherland 2005 had the smallest standard deviation in scores which are likely both due to decreased incidences of weathering which may have confounded colour scores in samples from the other locations.

As these markers are AFLP-based, they are not practical for use for high through-put screening such as would be required for a breeding program. It will be necessary to add sequence based markers (SSRs, SNPs, etc) to the map. This will be possible following mapping of the new lentil ESTs being carried out under a series of new projects being funded variously by ADF, SPG, ABIP and possibly Genome Canada with research partners at PBI and University of Guelph.

b) Plumpness

In 2007 we were able to grow sufficient quantities of seed from RIL populations segregating for seed plumpness to carry out rigorous phenotyping. Population LR44 (LC8602303T x 1125-1-5) was identified as best suited for mapping. Phenotyping was carried by sieving samples through round and slotted sieves as described above. A total of 47 individuals were phenotyped. Further growing seasons will be necessary to more accurately phenotype these RILs: multiple environments and multiple replicates make for much more accurate phenotypic data and identification of QTL relies on robust phenotypic data.

The parental lines 1125-1-5 and LC8602303T were screened with 28 SSR markers and 13 different AFLP primer combinations. Of the 28 SSRs, four were polymorphic between the parents and were used to genotype 82 individuals from the mapping population. Six AFLP primer combinations resulted in between seven and 14 polymorphic bands each and were used for mapping with 82 individual RILs. The rest were either monomorphic or too difficult to see distinct

polymorphisms. Linkage analysis on the mapping data resulted in the identification of seven linkage groups and 22 unlinked loci (Fig. 12).

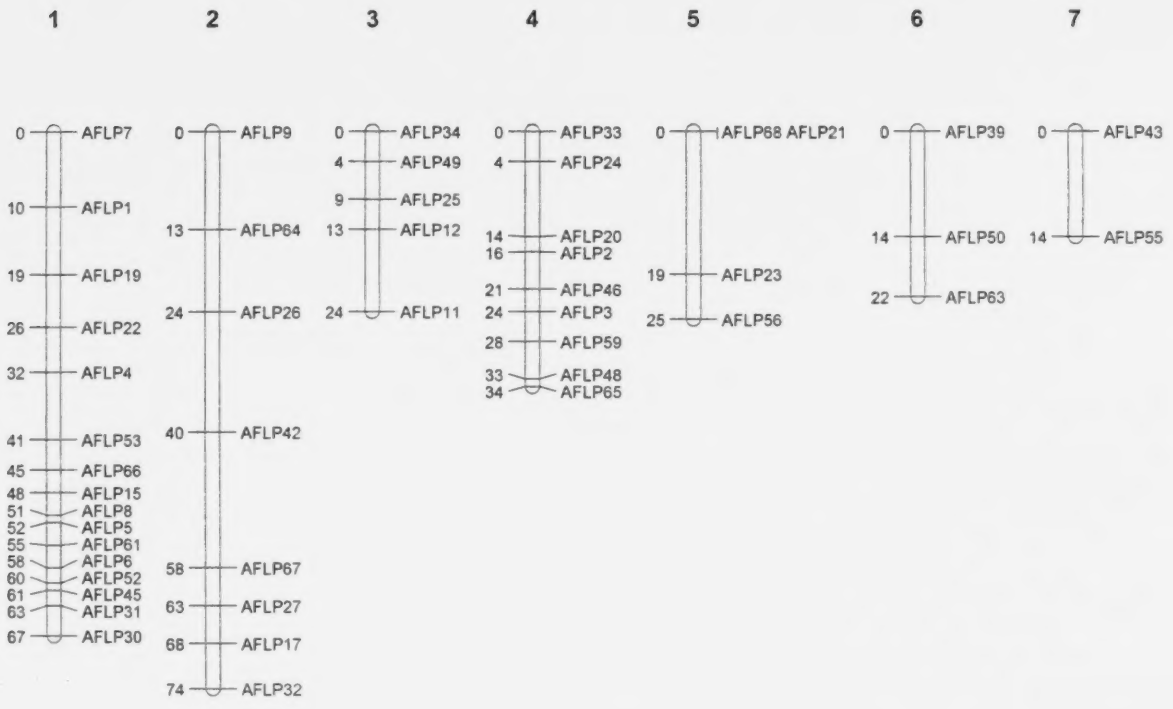

Figure 12. Genetic linkage map of LR44 (1125-1-5 x LC8602303T).

QTL analysis on this data set did not lead to the identification of any major QTL for shape based on any of the seven different methods of measuring it. This could be due to insufficient coverage of the genome with markers or inadequate phenotyping or both. By adding more markers to the map it may be possible to assign some of the unlinked markers to linkage groups and further extend some of the small groups. Rather than continue with anonymous AFLP markers, we intend to wait until gene-specific markers are available through recently funded projects in lentil molecular marker development (EST projects funded by ADF and ABIP and possibly Genome Canada). These markers will be much more useful as they will co-dominant (can identify heterozygotes) and tagged to specific lentil chromosomes.

A second look at the data sorted for each of the traits and then analysed using only the top and bottom 10 individuals revealed a region on linkage group 1 where there are significant differences in the allele found in the 10 individuals with the smallest diameter vs the 10 with the largest diameter (AFLP1 & AFLP19; $p = 0.046$). There was also a significant difference ($p = 0.027$) in the allele present at AFLP7, also on linkage group 1, for the 10 lines with the smallest thickness vs those 10 with the largest thickness. These regions will be worth noting in future mapping endeavors.

Presentations and Publications:

Vandenberg, A., D. Thavarajah, A. Tullu, J. Bruce, B. Davey, A. Fritzler, A. Sarker, M. Tahir, R. Chibbar and K. Bett. 2007. Genetic Improvement of Quality Traits in Lentil. European Grain Legume Association Conference, Lisbon, November.

Davey, B.F. 2007. Green Seed Coat Colour Retention in Lentil. M.Sc. Thesis. University of Saskatchewan.

Davey B.F., Vandenberg A., Van Natto C., Bett K. Green Seed Coat Color Retention in Lentil. Oral presentation at NAPIA, Madison WI, November 2007.

Fritzler, A. and Vandenberg, A. The Effects of Environment on Plumpness in Lentil (*Lens culinaris* Medik). Oral presentation at Department of Plant Sciences Undergraduate Thesis Conference, March 2007.

Davey B.F., Van Natto C., Bett K. and Vandenberg A. Effects of Swathing and Desiccation on Green Lentil Quality. Poster at Pulse Days, Saskatoon SK, January 2006.

Davey B.F., Vandenberg A., Van Natto C., Bett K. Green Seed Coat Color Retention in Lentil. Poster at 6th Canadian Pulse Crops Research Workshop, Saskatoon SK, Nov. 2006.

Davey B.F., Vandenberg, A., Van Natto, C., Bett, K. Colour Classification of Green Lentils. Poster and paper for NAPIA biennial meeting, Newark, Delaware, October 2005 and Pulse Days, Saskatoon SK, January 2006.

Information of benefit to producers, processors, or governments:

- It appears that swathing green lentil may result in a greener sample than does desiccation. Problems with disease and other quality issues that result from a swath lying out for extended periods during inclement weather should be considered though when deciding to swath vs desiccate.
- While the expression of green colour in the seed coats of green lentil is quantitative in nature, selection for better green colour appears to be possible given the right screening conditions. This should lead to improved green lentil colour in future varieties released for production in SK.
- Expression of seed plumpness is also quantitative but there is considerable genetic variability which means we should be able to select for more plump red lentils. This should lead to the development of new cultivars which could capture a premium for shape.
- We have implemented changes to the lentil breeding program based on the results of the project. We have changed the order of operation of seed cleaning to one in which segregating populations are screened first for thickness, followed by separation for diameter. We have also re-designed the screening and selection system for green lentil so that 1294M-23 has become a standard in all breeding nurseries. The line IBC-145 which is a backcrossed derivative of 1294M-23 with imidazolinone tolerance will be released as a

commercial cultivar to increase the potential for obtaining a premium value based on visual quality.

D. Personnel:

Field, lab and greenhouse technical support.

E. Equipment purchased or rented:

Lab and field equipment rental.
Growth facilities rental.
No major equipment purchased for this project.

F. Project Developed Materials
RIL populations used in this work are available for further characterization.

G. Project Photos
Some photos of various aspects of this work are available on request.

H. Acknowledgements:
The Saskatchewan Agricultural Development Fund and the Saskatchewan Pulse Growers has been and will be acknowledged in all presentations where this research project is mentioned.

I. Expense Statement:
Will be provided.

J. ICAR Data Entry
Will be entered.